International Scholars Journals

African Journal of Crop Science ISSN 2375-1231 Vol. 6 (10), pp. 001-010, October, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Use of a liquid inoculum of the arbuscular mycorrhizal fungi *Glomus hoi* in rice plants cultivated in a saline Gleysol: A new alternative to inoculate

F. Fernández[1,2], J. M. Dell'Amico[2], M. V. Angoa[3]* and I. E. de la Providencia[4]

[1]SYMBORG S.L. Campus de Espinardo No 7. Edificio CEEIM. Murcia. España. CP 30100.
[2]Instituto Nacional de Ciencias Agrícolas. (INCA), Habana, Cuba.
[3]Centro Interdisciplinario de Investigación para el desarrollo Integral Regional. (CIIDIR) IPN, Justo Sierra No. 28, CP 59519, Jiquilpán Michoacán, México.
[4]Université catholique de Louvain, Unité de microbiologie, 3 Place Croix du Sud, 1348 Louvain la Neuve, Belgium.

Accepted 09 March, 2018

The functioning and efficacy of a liquid inoculum containing spores of the arbuscular mycorrhizal fungi (AMF) *Glomus hoi* previously isolated from saline soil, were evaluated over growth and production of *Oryza sativa* plants cultivated under flooding conditions. Growth dynamics, grains weight, panicles number, as well as panicles weight were determined in the rice plants. Other mycorrhizal parameters such as biomass of ectophyte (EcB), arbuscular endophyte biomass (AEB), the ratio EcB:AEB, spore density and visual fungal density were also determined. The treatments evaluated were Mycorrhizal plants and Non-mycorrhizal plants (control). Results showed that rice plants inoculated with AMF grew higher than control plants. This was related to the AEB and EcB development. For the yield parameters the AMF treatment presented the highest values compared to control. These results showed the capability of a liquid inoculum to promote growth, development and improve the production of rice plants under saline conditions. This study showed the potential of using a liquid inoculum in field given its ability to store more spores without affecting their viability. In addition, the high applicability and easier manipulation of a liquid inoculum compared to the traditional solid inocula would reduce costs and maximize benefits in field.

Key words: Growth, increase production, *Oryza sativa*, liquid biofertilizer, saline soil.

INTRODUCTION

Oryza sativa is the second most important cereal in the world after wheat, and the principal crop in Asia, serving as food for about 50% of the world's population (Ladha et al., 1997; Sass and Cicerone, 2002). It is predicted that a 50 to 60% increase in rice production will be required to meet demand from population growth by 2025 (Zhang and Wang, 2005). World rice consumption increased 40 % in the last 30 years, from 61.5 kg per capita to about 85.9 kg per capita (milled rice) (UNCTAD- FAO, 2008). Protected horticultural crops as well as those planted in open fields such as rice, are coping with increasing salinization of irrigation water (Al-Karaki, 2006). This water contributes to generate more salinization of agriculture fields. Salinization is a process of soil degradation that is increasing in importance throughout the world (Keren, 2000; Liang et al., 2005).

Saline soils occupy more than 7% of the earth land surface and represent a major limiting factor in crop production (Feng et al., 2002; Nourbakhsh and Sheikh-Hosseini, 2006). Excessive amounts of salt have a range of adverse effects on the physical and chemical properties of soil, microbiological processes and plant growth (Yuang et al., 2007; Zhu, 2002). Salinization is often associated to poor fertility level of land (González-Nuñez et al., 2004). Plants photosynthesis, its efficiency

*Corresponding author. E-mail: valeangoa@hotmail.com. Tel: 3535330218. Fax: 3535330218.

and the rate of protein accumulation could be inhibited by low water and low fertilizer availability (Hasegawa et al., 2000). Nevertheless, the exploitation of soil microbes used in salt stressed lands or irrigated with saline water could be an alternative for plants development under these extreme conditions (Giri et al., 2003). It has been demonstrated that mutual associations of plants with soil microorganisms may improve plant tolerance to stressful conditions (Carvalho et al., 2003).

Several studies have shown that inoculation with arbuscular mycorrhizal fungi (AMF) can diminish the stress caused by salinity (Al-Karaki et al., 2001; Azcón-Aguilar and Barea, 1997; Dixon et al., 1993; Hartmond et al., 1987; Juniper and Abbott, 1993; Rao, 1998; Ruiz-Lozano et al., 1996; Singh et al., 1997). AMF improvement of salt resistance has been usually associated with AM-induced increases in phosphorous acquisition and plant growth (Rosendahl and Rosendahl, 1991), as well as in the resistance of the crop (Al-Karaki, 2000; Cho et al., 2006). Ruiz-Lozano et al. (1996) and Azcón and El-Atrash (1997), suggested that plants could be more effectively protected against salinity stress by AMF symbiosis rather than by phosphorous supplementation. However, responses to deficiency of oxygen in soil, such as a decline in photosynthetic capacity, stomatal conductance and nutrient uptake (Kozlowski and Pallardy, 1984), may induce a lower allocation of carbohydrates from the host plant to the fungi (Carvalho et al., 2003).

It is known that wetland plants can increase the flow of oxygen to their rhizospheres through aerenchyma development (Chabbi et al., 2000). It is likely that the aerobic AMF in wetlands obtain oxygen via this route, which could also depend partially on air spaces in the cortex (Carvalho et al., 2003; Ipsilantis and Sylvia, 2007; Smith and Smith, 1997). In addition, there is a limited knowledge of AMF interactions with wetland plants although its presence is well known (Khan and Belik, 1995; Thormann et al., 1999). Apparently, there is no relationship between the percentage of root length colonized by AMF and the plant hydrological category (Aziz et al., 1995; Turner et al., 2000).

Flooding conditions may suppress the mycorrhizal association (Miller, 2000) but do not affect root colonization (Ipsilantis and Sylvia, 2006; Stevens and Peterson, 1996). An oxygen restriction could either delay the extension of external mycelium into flooded soil (Beck-Nielsen and Madsen, 2001) or promote the adherence of high amounts of hyphae to the roots (Hildebrandt et al., 2001). However, knowledge on the extent to which salinity and flooding affect the growth and efficacy of the indigenous AMF is scarce (Carvalho et al., 2003). Feng et al. (2002) showed that selected AM species increased the resistance to osmotic stress due to a significant increase in soluble sugars and electrolyte concentration in maize roots. Ruiz-Lozano and Azcón (2000) reported an improvement in the salinity tolerance of lettuce plants inoculated with AMF and a specific strain of *Glomus* isolated from saline conditions. This inoculum protected the plants against the detrimental salt effects through an increase on the radical system.

Although there are some reports about inoculation of AMF in rice crops under field conditions (Fernández et al., 1997; Secilia and Bagyaraj, 1992), its use is really scarce and it always involves a solid substrate to pelletize the seed (Rivera and Fernández, 2005). The solid substrate technique is useful; nevertheless, it is complex due to the great volume of seeds needed to sowing (150 and 200 kg $\cdot ha^{-1}$). According to the variety of plant, soil and fertilization program, great machines would be necessary to make the inoculation process in a homogeneous way; therefore, the use of a liquid inoculum could represent a more convenient option to ease inoculation in field.

Glomus hoi has been characterized by a high production of external mycelium, spores, frequent arbuscules even at the end of crop (Fernández et al., 2006a); it has been developed in different host plants as *Sorghum vulgare* and *Brachiaria decumbens*. But its potential for being used to counteract stress caused by salinity is a derivative of the fact that this fungus has always been used in saline soils with a high electric conductivity of 8315 µS cm^{-1} and sodium contents of 6.62 cmol kg^{-1} soil (Dell´Amico et al., 2007).

The objective of this work was to evaluate whether the inoculation of rice plants with AMF species isolated from a heavily saline soil could improve their development and performance under saline edaphic conditions; additionally we tested the efficacy of a liquid inoculum of AMF, *G. hoi* rather than a solid traditional inoculum to investigate a potentially more efficient alternative.

MATERIALS AND METHODS

Description of the study area

This work was conducted in the central Greenhouse of the National Institute of Agricultural Sciences (INCA), in San Jose de las Lajas, Havana, Cuba which is located at the geographic coordinates: 23° 0.12 .06 "N Latitude and 82° 8'31 .46" W Longitude.

Soil

Soil used in the experiments was a Gleysol containing: 2.3% organic matter, 0.5 g kg^{-1} total nitrogen, 13.2 cmol kg^{-1} total P, 10.2 cmol kg^{-1} of calcium, 5.6 cmol kg^{-1} of magnesium, 0.9 cmol kg^{-1} of potassium, 6.9 cmol kg^{-1} of sodium, an electric conductivity of 8789 ųS cm^{-1} and a pH of 8.2 in H_2O.

Mycorrhizal fungi inoculums

G. hoi (Berch and Trappe) previously isolated from pasture under saline soils (Fernández et al., 2010) at Departamento del Chaco, Bolivia, was used in this study. It was selected as an efficient isolate for improving plant growth under salinity stress conditions

Table 1. Plant height (cm), root system length (cm) and mycorrhizal colonization (%) quantified during the 90 days after germination (DAG) in AMF-treated and control plants.

DAG	1	5	10	20	25	35	40	60	90
					Plant height (cm)				
AMF	10.5 b	10.5 b	10.9 b	15.1 a	19.8	23.8 a	32.8 a	63.5 a	74.1 a
Control	11.1 a	11.2 a	12.2 a	17.6 b	18.9	20.7 b	25.7 b	58.6 b	68.1 b
Es x	0.2***	0.12***	0.1***	0.2***	0.9 ns	0.1***	0.2***	0.6***	0.2***
					Root length (cm)				
AMF	0.6	0.6	1.4 b	6.18b	6.82b	7.9 a	17.08a	23.9 a	26.9 a
Control	0.6	0.6	2.1 a	6.29a	6.4a	6.4 b	7.23b	10.4 b	13.1 b
Es x	0.0 ns	0.3 ns	0.2***	0.3 ns	0.4 ns	0.2***	0.3 ns	0.1***	0.2***
					Mycorrhizal colonization (%)				
AMF	2 a	6 a	13 a	17 a	18 a	24 a	35 a	38 a	44 a
Control	0 b	3 b	3 b	12 b	13 b	15 b	20 b	19 b	22 b
Es x	0.3***	0.2**	0.13***	0.1***	0.2***	0.1***	0.2***	0.2***	0.4***

Values within a column followed by the same letter do not differ significantly (P<0.05) using a Tukey HSD test n=10.

(Fernández et al., 2006b); inoculum was propagated using *S. vulgare* for four months. Spores were isolated from substrate by a sedimentation process. First, 250 g of substrate and propagules were mixed with water in a tank by mechanical stirring for 15 min. This mix was decanted in a filtration cylinder to collect the supernatant.

Supernatant was centrifuged at 2000 g for 5 min to obtain the spores along with the mycelium fragments and it was stored in a protective osmotic solution (number of international publication of patent WO 2006/060968 AI) (Fernández et al., 2006c).

Evaluation of the mycorrhizal inoculum efficacy on growth and production of plants

The experiment was conducted in a greenhouse at a temperature range of 20 to 35°C. Soil (1000 g per pot) was placed into 20 × 15 ×15 cm plastic pots. 15 seeds of *O. sativa* L (cv.LP5) were sown into each container. Each pot was considered a single sample, and there were ten sample replicates per treatment. Treatments were divided into:

1) Non-mycorrhizal control; and
2) Inoculation with a liquid mycorrhizal application.

The mycorrhizal treatment was inoculated with a 100 ml of the protective osmotic solution that contained 3000 spores and mycelium fragments whereas the non-mycorrhizal treatment was rinsed with 100 ml of the same protective osmotic solution free of mycorrhizal spores. An amount of 100 ml of a nutrient solution containing 100 mg L^{-1} of nitrogen, 50 mg L^{-1} of phosphorous and 50 mg L^{-1} of potassium was added to the soil every week (Jeon et al., 2004; Minagri, 1999). Tap water was supplied daily during 20 days after sowing, and a water film was implanted during the entire assay to maintain the flooding conditions.

Plant height, root length and mycorrhizal activity (root colonization, visual fungal density, spore density and fungal biomass) of five plants per pot per treatment were measured at day 1, 5, 10, 15, 20, 25, 40, 60 and 90. Note that mycorrhizal activity could be measured at day 1 due to the presence of residual mycelium and roots remaining in the suspension inoculated.

These propagules established communication with the seed and started the colonization through the issuance of both extramatric and internal mycelium at the following days. It has been observed that the biochemical activity between inoculum and host plant could be developed 24 h after inoculation (Pérez-Ortega, 2010). After 90 days, the plants were harvested and the yield components (panicle length, spikelet number per panicle, and grain yield) were quantified. The experiment was based on a completely random design. A one-way analysis of variance (ANOVA) was performed in order to determine if there were significant differences between treatments. Post-hoc comparisons were then analyzed using a Duncan's multiple-range test ($p<0.05$).

Analyses of mycorrhizal colonization and visual fungal density

Mycorrhizal colonization and the visual fungal density were tested according to the grid line-intersect method described by Giovannetti and Mosse (1980) and Trouvelot et al. (1986). Fine roots were collected and washed with water. The washed roots were cut into 1 cm segments and thoroughly mixed. A sub-sample of 0.5 g was bleached with 10% (w/v) KOH at 90°C for 2 h and stained with trypan blue (Phillips and Hayman, 1970).

Quantification of ectophyte/arbuscular endophyte biomasses and total spore density

Ectophyte biomass (EcB) and the total spore densitiy in soil were analyzed based on the method by Herrera-Peraza et al. (2004). The ratio EcB: arbuscular endophyte biomass (AEB) was calculated as an indicator of mycorrhizal function. Each experiment was repeated three times.

RESULTS

Efficacy of the mycorrhizal inoculum on growth and production of plants

Plant growing

The application of AMF liquid inoculum had a positive effect on the growth dynamics of plants and produced a remarkable effect on the variables analyzed (Table 1).

Table 2. Yield and some of its components: Panicles.plant^{-1} (P), panicles weight (PW) and 100 grains weight (GW) determined in AMF-treated and control plants.

Treatments	P	PW (g)	100 GW (g)	Yield (g plant^{-1})
AMF	8.33 a	2.70 a	3.69 a	21.66 a
Control	5.40 b	1.87 b	2.70 b	15.40 b
Es x	0.12 ***	0.05***	0.001***	1.34***
C.V (%)	11.2	9.2	6.5	14.6

Values within a column followed by the same letter do not differ significantly (P<0.05) using a Tukey HSD test, n=10.

Nevertheless, up to 20 days, the highest growth values corresponded to the control plants; whereas at the end of their life cycle, mycorrhizal-inoculated plants performed significantly better than the control group ($p< 0.05$). Differently, the root growth was very slow and little compared to the high of the stem. At an early stage, both groups showed a similar rate of growth; at day 10 the control root was longer than the mycorrhizal-inoculated treatment and this effect was maintained for 25 days. Roots of inoculated plants had a significant two fold-increase lengthwise compared to the controls ($p< 0.05$).

The main yield components for both treatments of rice plants are shown in Table 2. An increase in yield and its components in the AMF treatment was noteworthy, as a clear sign of the efficiency of this type of fungal inoculum on the plant productivity even under saline conditions.

Root colonization, visual fungal density and spore density

The analysis of AMF colonization was a progressive process and a high value was obtained (Table 1). In inoculum-free plants there was a poor colonization because of the unsterilized soil used in this experiment (data not shown). Since root colonization only indicates the presence of the mycorrhizal fungi hyphae in the root but not the fungal intensity in the inner root, a fungal visual density test was conducted according to the method reported by Trouvelot et al. (1986) This test expresses more adequately the efficacy of the mycorrhizal fungi to promote growth and production in the plants. The fungal occupancy had a typical microbial behavior where the symbiont slowly colonized the inner root from 0 up to 20 days (Figure 1). An exponential growth of mycelium with a latent phase very well defined was observed after 40 days, when the fungal activity reached a stationary phase to the end of crop. This behavior was related with the spore dynamics (Figure 1).

During the first day, a low number of spores appeared in the soil samples, as a cumulative result of the pool of spores from the initial inoculation. This number increased along the next days, due to the germination process in adequate conditions of humidity and temperature. Accumulation of the new spores production began at 30 days, as a direct consequence of the increase in the production of mycelium and the symbiosis development. The increment of the spore population continued to grow up until reaching 12 spores g soil^{-1} (Figure 1).

EcB and AEB

EcB and AEB (Figure 2) are some of the main indicators of the fungal symbiont presence in annual crops, due to the strong relationships between their functioning and the plant symbiosis process. The biggest EcB was presented during the first development stages of symbiosis. In contrast, the arbuscular endophyte developed low biomass content over the first 25 days, after which, high values were obtained. This stage could be considered as a transition phase. After 30 days, the EcB decreased but then achieved a stabilization process. AEB had a different performance: a gradual increment associated with plant growth and symbiosis development. The EcB:AEB ratio (Figure 3), indicates the actual mycorrhizal functioning (Fernández, 2003). The performance was initially characterized by a strongly decreasing curve, which means a high EcB and a low endophyte presence. This was the result of hyphal spread in soil from host root.

After 20 days, a slight transitional stage takes place and a mutualistic phase was immediately implanted up to the end of the crop. Once the inner fungal components were in equilibrium with the ectophyte, the biomasses values fell down to below one, or even lower. From our point of view, this kind of mycorrhizal performance under this saline soil conditions have two marked phases:

First, an initial parasitic-transitional phase (from 0 to 25 days), with a great amount of ectophyte presence; second, a phase characterized by an intense endophyte presence and significant plant growth (starting from 25 days).

DISCUSSION

Plant growth

The plant growth in response to AMF colonization varied

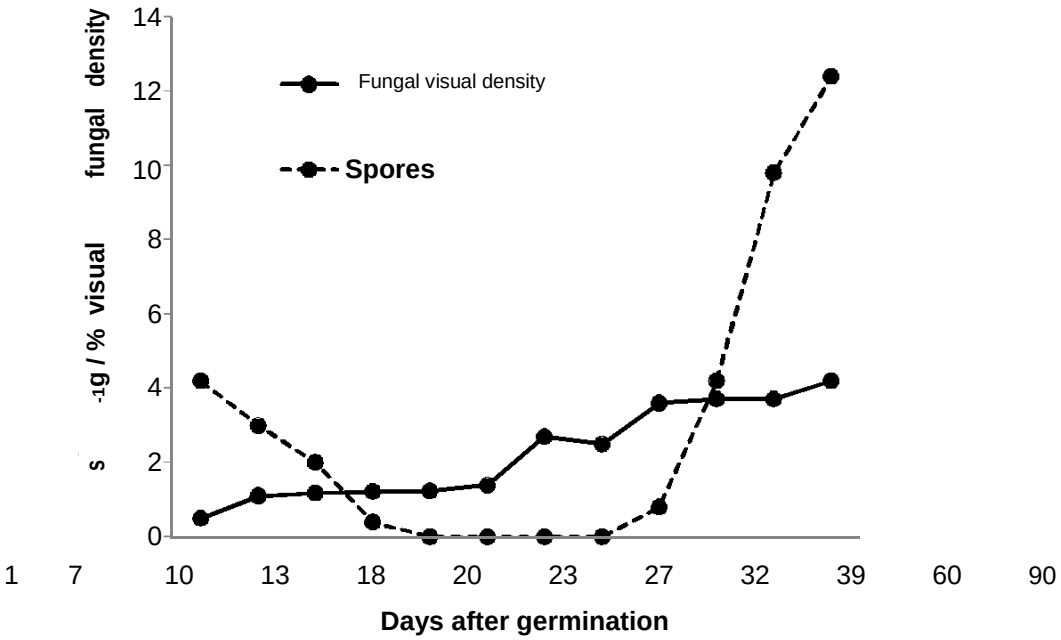

Figure 1. Spore dynamics and fungal visual density (%), in rice plants inoculated with liquid AMF under saline soil.

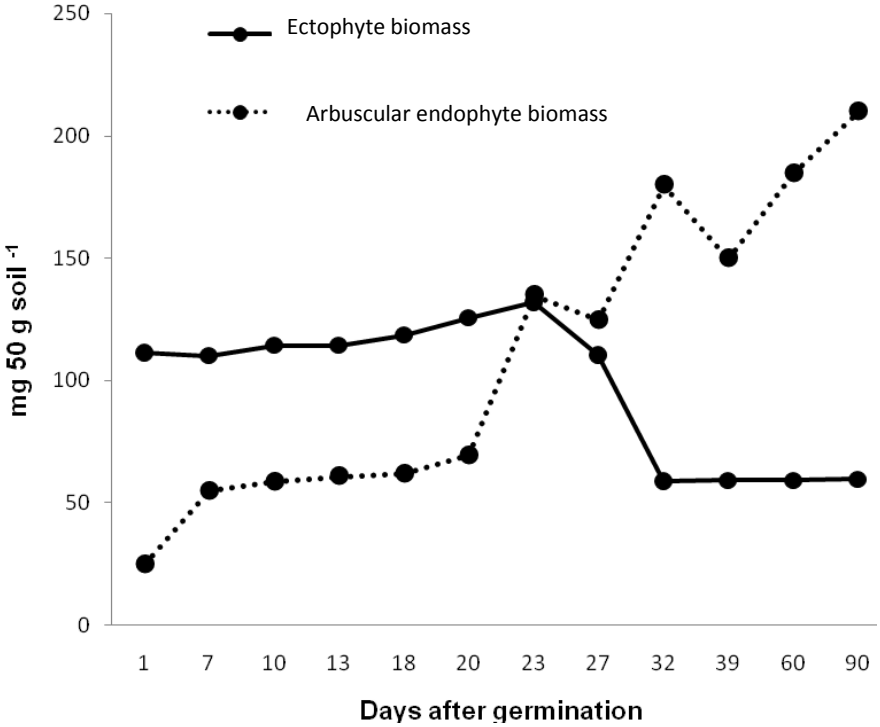

Figure 2. Dynamics of the Endophyte biomasses (EcB) and the Arbuscular ectophyte biomasses (AEB) for 100 days after germination in rice plants under saline soil, inoculated with a liquid AMF suspension under saline soil.

widely in its timing (Table 1). In this work this response was observed after 20 days and it was sustained until 90 days, in contrast to the reported by Bethlenfalvay et al. (1982), who found in soybean that the response was 42

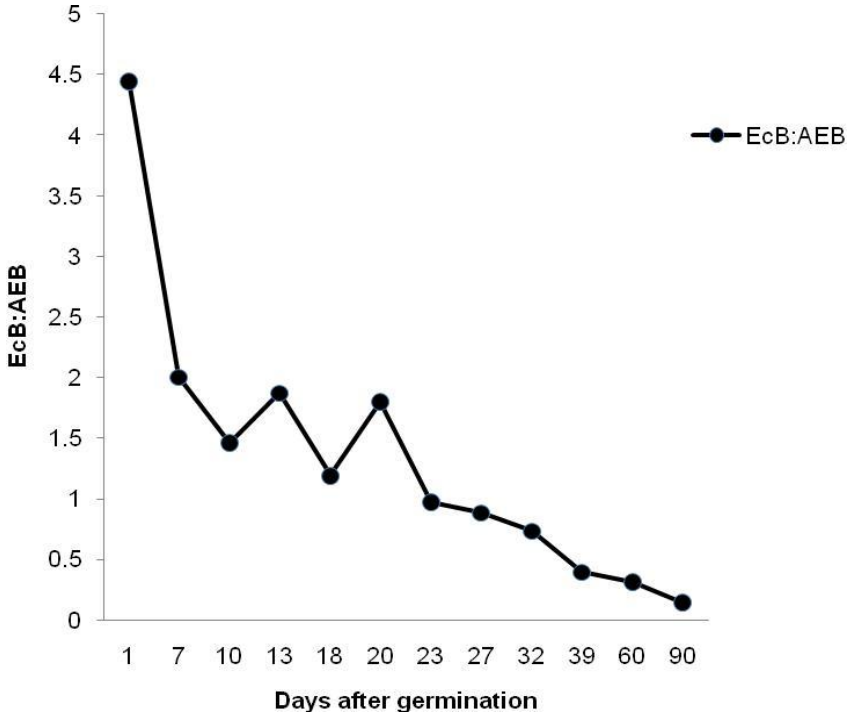

Figure 3. Ectophyte biomass (EcB): Arbuscular endophyte biomass (AEB) ratio found in rice plants inoculated with a liquid AMF suspension under saline soil.

days after planting and the growth was delayed 70 days. The promoter effect of AMF on the height and development of aerial part and the root system of the plants have been reported by several authors (Bethenfalvay and Linderman, 1992; Fernández et al., 1997; Fernández, 1999; Llonin and Medina, 2002; Terry et al., 1998). Indeed, salts could affect the capacity of the plant to supply carbohydrates, required for hyphal growth, by decreasing photosynthesis and plant growth under salinity (Mc Millen et al., 1998; Munns et al., 1995). However, beneficial effects of AMF on plant growth were observed after the establishment of the symbiont. This response could be close related to the mycorrhizal effectiveness, as the symbiosis process involves fluxes of photosynthates to the root system. Therefore, an adequate mycorrhizal development due to the use of these substances implicates a strong root system (Bonfante and Perotto, 1992; Bowen, 1987).

Results reported by Debouba et al. (2006) showed a greater shoot and root dry matter in tomato plants pre-inoculated with AMF irrigated with both saline and non saline water. Similar shoot and root weight increases were reported in banana (Yano-Melo et al., 2003), cotton (Tian et al., 2004), zucchini (Colla et al., 2007), *Lotus glaber* (Sannazzaro et al., 2006), soybean (Sharifi et al., 2007) and *Acacia auriculiformis* (Giri et al., 2003). However, our results differed from those studies in showing that the shoot was higher than the root (Table 1). Debouba et al. (2006) found that the increase of the root/shoot ratio in tomato was due to the higher sensitivity to salt of the leaves compared to the roots. The increase in this root/shoot ratio supports the hypothesis that the tomato assigns more dry weight to roots in order to maximize its capabilities for nutrient and water absorption. This is consistent with the results of Zhang (1995) who found that an increase in the root/shoot ratio reduced the shoot growth and yield, as well as the plant efficiencies in the use of water and nutrients (Debouba et al., 2006). This may also indicate that salt conditions induced nutritional deficiencies (Shangguan et al., 2004).

Studies in rice plants showed a bigger increment in the height of the shoot compared to the root (Maggio et al., 2004). This could be explained as a result of an inhibition of the root growth induced by salinity (Maggio et al., 2004). However, it was found in tomato plants that the maximum root density was reached at 60 days after transplanting and it gradually decreased until harvesting (Al-Karaki 2006). Our results show that the increment in rice growth started at 20 days after transplant, and it continued until 90 days. It is clear that salinity did not increase plant mortality, but it caused a reduction of root development (Table 1). In this sense, the response of inoculated rice to salinity was higher than the control. In the most commonly cultivated rice areas, young seedlings are very sensitive to salinity (Flowers and Yeo, 1981; Heenan et al., 1988). However, our data show a high production of rice grains (21.66 g plant^{-1}) compared to the control (15.40 g plant^{-1}) ($p<0.05$), as can be

observed in all yield components related to final grain (Table 2).

Similar results were found in zucchini fruits where mycorrhizal colonization enhanced the fruit dry matter with the highest values recorded on inoculated plants (average 5.9%) compared to non-treated plants (average 5.6%). It is well established that crop growth and yield decrease with high salinity (Colla et al., 2007). AMF alleviate the detrimental effect of salinity on growth and productivity. Improved nutritional and leaf water status might have assisted the plants to translocate minerals and to assimilate them as well as to alleviate the impacts of salinity on fruit production (Colla et al., 2007). Contrary to the studies reported by other researchers who found that yield was severely affected by salinity, in this work was observed that some parameters such as panicle length, spikelet number per panicle, and grain yield were significantly improved in salt treatments (Table 2) (Cui et al., 1995; Heenan et al., 1988; Khatun et al., 1995; Zeng and Shannon, 2000). Salinity also delayed the panicle emergence and flowering (Khatun et al., 1995). These results show the positive effect of the mycorrhizal inoculation to improve growth under these unfavourable conditions. This is consistent with the observed by Al-Karaki (2006) in pre-inoculated tomato plants with AMF irrigated with saline water, where yield (5.26 kg m^2) and fruit fresh yield (23 g) were greater than in non-inoculated plants (3.28 kg m^2 and 15 g respectively). This improvement in fruit fresh yield due to AMF inoculation was 29% under non saline and 60% under saline water conditions (Al- Karaki, 2006).

Some authors have found crops with low specificity for certain AMF strains in a specific soil condition (Fernández, 1999; Fernández-Martín et al., 2004; Rivera and Fernández, 2005; Ruiz, 2001; Sánchez, 2001). It points out the need of a competent mycorrhizal strain to establish an efficient colonization with any mycorrhizal-dependent crop. Indeed, crops show different quantitative effects to the mycorrhizal inoculation, but the competent strains could be used in the majority of crops. In this regard, several authors had found a strong mycorrhizal dependency in maize and cotton plants using the fungal strain *Glomus mosseae* under saline conditions (Feng et al., 2002). Cotton plants inoculated with different isolates of *G. mosseae* increased significantly their shoot dry weight by 68% under 1 to 3g NaCl kg^{-1}, and by 27% under 2g NaCl kg^{-1}.

Nevertheless, their shoot dry weight improved significantly by 31% when the NaCl level was 3g kg^{-1}, while root growth was not significantly affected (Tian et al., 2004).

Root colonization, fungal visual density and spore density

Structures characteristic of AMF were observed in the roots after inoculation at all levels of salinity (Figure 1). The percentage of root colonization (Figure 1), was higher in mycorrhizal than non-mycorrhizal plants, similar to the observed by Giri et al. (2003). This plant dependence seems to have a significant ecological importance, related to the adaptation of AMF to the saline stressful conditions. The fungal strain used in this assays, was isolated from a saline soil. It seems that the performance of the particular AM species under saline conditions is closely related to its adaptability to the ecological niches, the places, the original conditions of isolation and the substrates where it has been preserved up to this use. The AMF could follow a strategy to keep the functional memories in the symbiotic capacity to be used under similar conditions to the ones where it was originally found.

Since AMF require oxygen to succeed, stressful environments regularly flooded with saline water may be detrimental for their survival and infectivity (Saint-Etienne et al. 2006). Nevertheless, some AMF are able to persist in flooded soils and to colonize wetland plants (Khan, 1993; Landwehr et al., 2002; Miller and Bever, 1999; Turner et al., 2000). More than 50% of the plant's populations have been colonized by AMF in some wetlands conditions (Ragupathy et al., 1990). Brown and Bledsoe (1996), observed AMF in the aerenchymatous tissue of saline marsh plants, suggesting that AMF are adapted to life in oxygen-deficient soils.

Results observed in rice related to an increase in the ratio of root growth of AMF treatment with the increase in water level could be a result of development of an aerenchyma, such as the one reported by Carvalho et al. (2003) for *Aster tripholium* plants. These particular plants have the capability to induce a well-developed aerenchyma, suggesting that AMF could thus overcome the lack of oxygen in soil by colonizing these more oxygenated portions of the root. Therefore the effect of flooding on fungal growth was greater in EcB than in AEB (Figure 3). If we take into account that the assay was performed under flooding conditions starting from 15 days, it could be supported that mycorrhizal colonization was very effective. At the end of the life cycle of the crop a root colonization percentage of 44 could be observed. We considered it elevated compared to other experiments in rice with inocula in solid base such as MicoFert and EcoMic, where the maximum mycorrhizal colonization values were around 25% (Fernández et al., 1997).

Similar results were reported by Sannazzaro et al. (2006) who found a reduction in mycorrhizal fungus colonization of *Lotus glaber* plants grown under saline conditions. This could be explained as it has been shown that AMF may be affected by salinity during spore germination (Juniper and Abbott, 1993), hyphal growth (McMillen et al., 1998) and arbuscule formation (Pfeiffer and Bloss, 1988). However, the increment in root colonization in rice in presence of the liquid inoculum has

been already observed by others authors in tomato, lettuce and maize, as well as in different type of soils (Sánchez –Blanco et al., 2004) and (Fernández et al., 2006a). Native spore population was low (less than 1g $soil^{-1}$), very typical of agricultural saline soil with high sodium content and other salinity conditions in which the AMF presence and diversity always decreases because of the intensive till and overexploitation, among others (Rao, 1998).

The AMF spore production has been well documented in the literature, especially in *Glomus* species, which are great spore producers. Studies are facilitated because of the fungal development in the arbuscular-mycorrhizal associations and the fungal life cycle. In our study, large amounts of spores could be obtained under conditions of inoculum production. Once the vegetable tissue is dead, the spore contents can be multiplied up to twice due to the fast translocations of nutrients from roots to the hyphal network in order to form new ones. Values obtained for *G. hoi*, the species used in this assay, were nearest to 300 spores g $substrate^{-1}$ (Fernández, 2003).

EcB and AEB

The increment in the spore production could be related to the biomasses of the ectophyte and endophyte (Figure 2). Initially, there was a rather more external mycelium than inner one but this condition was reversed after day 20. At the final harvest, endophyte biomass was almost three times bigger than the external component. These results were similar to the data reported by Bethlenfalvay et al. (1982). The biggest EcB presented during the first development stages of symbiosis (Figure 2), was caused by the parasitic fungal abilities to live at expenses of the plant, as previously reported by other authors as a parasitic phase (Bethlenfalvay et al., 1989). This phase is considered a derivative of the exuberant fungal mycelium growth at early stages of plant growth with a low photosynthetic rate and a high metabolic cost.

AEB (Figure 2) had a different performance: a gradual increment associated with plant growth and symbiosis development

Some authors have previously described that process using fungal strains in solid substrate (Bethenfalvay and Linderman, 1992; Fernández, 2003). Therefore, this work is the first report where AMF conserved in a liquid suspension has been used. The EcB: AEB ratio (Figure 3) was characterized by a strong declining curve, which means a high EcB and low endophyte presence. This was provoked by hyphal spread in soil from host root to explore the surrounding environment looking for nutrients for itself to maintain the symbiosis with plant and to increase the reciprocal interchanges with the host tissue. This particular behavior has been recently described under *in vitro* conditions as a common event (Bago and Cano, 2005). A diminished plant growth was also observed during early stages up to 18 to 20 days, as a clear sign of the efforts that plants need to do in order to maintain the symbiosis (Table 1). An evident increase in the AEB was presented derived from the high nutrient demand and the arbuscular increase in the inner cell host (Figure 2).

Previous studies had reported that salinity reduced the mycorrhizal colonization, caused an inhibition of germinative power in spore of genus *Glomus*, decreased the ectophyte growth in soil (Hirrel, 1981; Rao, 1998), reduced the normal spread of hyphal network after the colonization (McMillen et al., 1998) and decrease the arbuscular number in host plant (Pfeiffer and Bloss, 1988). This work showed that the inoculation with a liquid suspension of AMF abolished the major limitations blocking the fungus development and the subsequent nutritional benefits conferred to the rice plants to which it was associated. We can point out that the use of this type of liquid inoculum as well as the AMF strain were very effective under soil condition where high salinity and deficient nutrients are conjugated to act in a negative way on the plant growth. Moreover, the use of a liquid inoculum along with an efficient AMF that shows an intensive response under adverse conditions is a feasible option and opens new strategies to be used as tools for improving the development of crops under stress conditions.

ACKNOWLEDGEMENTS

We would like to thank “Instituto de Ciencia y Tecnologia de Cuba” for funding this work and Instituto Nacional de Ciencias Agrícolas (INCA) de la Habana, Cuba for lending us their facilities to conduct this research. We thank Professor David Thomas for critically and exhaustively reviewing this manuscript.

REFERENCES

Al-Karaki GN (2000). Growth of mycorrhizal tomato and mineral acquisition under salt stress. Mycorrhiza, 10: 51–4.

Al-Karaki GN, Hammad R, Rusan M (2001). Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. Mycorrhiza, 11: 41–47.

Al-Karaki GN (2006). Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Sci. Hortic., 109: 1–7.

Azcón-Aguilar C, Barea JM (1997). Applying mycorrhiza biotechnology to horticulture: significance and potentials. Sci. Hortic., 68: 1–24.

Azcón R, El-Atrash F (1997). Influence of arbuscular mycorrhiza and phosphorus fertilization on growth, nodulation and N_2 (N-15) in *Medicago sativa* at four salinity levels. Biol. Fert. Soils, 24: 81–6.

Aziz T, Sylvia DM, Doren RF (1995). Activity and species composition of arbuscular mycorrhizal fungi following soil removal. Ecol. Appl., 5: 776–784.

Bago B, Cano C (2005). Breaking myths on Arbuscular Mycorrhizas in vitro biology. In: Declerk S, Strullu DG and Fortin JA (Eds). *In vitro* culture of Mycorrhiza. Soil Biol. Springer-Verlag, Berlin Heidelberg. Pp. 111-138.

Beck-Nielsen D, Madsen TV (2001). Occurrence of vesicular–arbuscular mycorrhiza in aquatic macrophytes from lakes and streams. Aquat. Bot., 71: 141–148.

Bethlenfalvay GJ, Pacovsky RS, Brown MS, Fuller G (1982).

Mycotrophic growth and mutualistic development of host plant and fungal endophyte in an endomycorrhizal symbiosis. Plant Soil, 68: 43-54.

Bethlenfalvay GJ, Brown MS, Franson RL, Mihara KL (1989). The glycine-*Glomus-Bradyrhizobium* symbiosis - IX. Nutritional, morphological and physiological response of nodulated soybean to geographic isolates of the mycorrhizal fungus of *Glomus mosseae*. Physiol. Plant, 76: 226–232.

Bethenfalvay GJ, Linderman JA (1992). Mycorrhizae and crop productivity. Horticultural Crops Research Laboratory. USDA-ARS.

Bonfante-Fassolo P, Perotto S (1992). Plants and endomycorrhizal fungi: The cellular and molecular basis of their interaction. In: Verma DP (Eds). Molecular signals in plant-microbe communications. CRC press, Boca Raton, pp: 445–470.

Bowen GD (1987). The Biology and Physiology of infection and its development. In: Safir GR (Ed), Ecophysiology of VA Mycorrhizal Plants. CRC Press, Boca Raton, pp. 27-57.

Brown AM, Bledsoe C (1996). Spatial and temporal dynamics of mycorrhizas in *Jaumea carnosa*, a tidal saltmarsh halophyte. J. Ecol., 84: 703–715.

Carvalho LM, Correia PM, Caçador I, Martins-Louçao MA (2003). Effects of salinity and flooding on the infectivity of salt marsh arbuscular mycorrhizal fungi in *Aster tripolium* L. Biol. Fert. Soils, 38: 137–143.

Chabbi A, Mckee KL, Mendelssohn IA (2000). Fate of oxygen losses from *Typha domingensis* (Typhaceae) and *Cladium jamaicense* (Cyperaceae) and consequences for root metabolism. Am. J. Bot., 87: 1081–1090.

Cho K, Toler H, Lee J, Ownley B, Stutz JC, Moore JL, Augé RM (2006). Mycorrhizal symbiosis and response of sorghum plants to combined drought and salinity stresses. J. Plant Physiol., 163: 517-528.

Colla G, Rouphael Y, Cardarelli MT, Tullio M, Rivera CM, Rea E (2007). Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. Biol. Fert. Soils, pp. 1-9.

Cui H, Takeoka Y, Wada T (1995). Effect of sodium chloride on the panicle and spikelet morphogenesis in rice. Jpn. J. Crop Sci., 64: 593–600.

Debouba M, Gouia H, Suzuki A, Ghorbel MH (2006). NaCl stress effects on enzymes involved in nitrogen assimilation pathway in tomato ‘‘*Lycopersicon esculentum”* seedlings. J. Plant Physiol., 163: 1247-1258.

Dell'Amico JM, Fernández F, Nicolás E, López LF, Sánchez-Blanco MJ (2007). Physiological response of tomato to the application of twoinoculants *Glomus sp1* (INCAM 4) by two different routes of inoculation. Trop. Crops, 28: 33-38.

Dixon RK, Garg VK, Rao MV (1993). Inoculation of *Leucaena* and *Prosopis* seedlings with *Glomus* and *Rhizobium* species in saline soil: Rhizosphere relations and seedlings growth. Arid Soil Res. Rehabil., 7: 133–144.

Feng G, Zhang FS, Li XL, Tian CY, Tang C, Rengel Z (2002). Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza, 12: 185–190.

Fernández F, Ortiz R, Martínez MA, Costales A, Llonín D (1997). The effect of commercial arbuscular mycorrhizal fungi (AMF) inoculants on rice (*Oryza sativa*) in different types of soils. Cult, Trop., 18(1): 5-9.

Fernández F (1999). Managing arbuscular mycorrhizal associations on the production of coffee seedlings (*C. arabica* L. var. Catuaí) in some soil types, [Thesis Phd.], INCA, p. 128.

Fernández F (2003). Advances in the production of arbuscular mycorrhizal inoculants. In: Rivera R and Fernández K (Eds), Effective management of themycorrhizal symbiosis, a path to sustainable agriculture. Caribbean Case Study. Foreign Ministry in Havana, Chapter IV, pp. 144-166.

Fernández-Martín F, Rivera Espinosa RA, Providencia I, Fernández K, Rodríguez Y (2004). Effectiveness of mycorrhizal inoculation by seed dressing. Fungal functioning and agrobiological effect. In: Frías-Hernández JT, Olalde-Portugal V and Ferrera-Cerrato R (Eds), Advances in the understanding of the biology of mycorrhizae. University of Guanajuato, ISBN: 968-864-333-5, pp. 252-267.

Fernández-Martín F, Dell´Amico RJM, Rodríguez P (2006a). Effectiveness of some types of mycorrhizal inoculants in tomato crop. Trop. Crops, 27: 25-30.

Fernández-Martín F, Gómez Alvarez R, Venegas LLF, Martínez SMA, de la Noval PBM, Rivera ERA (2006b). Mycorrhizal inoculant product. Patent CU 22641 A1. Oficina Cubana de la Propiedad Industrial (OCPI), Available at: http://www.ocpi.cu/doc/2002/t7696.pdf, viewed 5 April.

Fernández F, Dell'Amico JM, Pérez Y (2006c). Liquid Mycorrhizal Inoculant. http://wipo/int/pctdb. Patente WO/2006/060968.

Fernández F, Dell Amico JM, Alarcón JJ, Nicolás E, Pedrero F (2010). Improving rice yield through the application of mycorrhizal isolated in saline conditions. Agriculture, 935: 916-919.

Flowers TJ, Yeo AR (1981). Variability in the resistance of sodium chloride salinity within rice (*Oryza sativa* L.) varieties. New Phytol., 88: 363–373.

Giri B, Kapoor R, Mukerji KG (2003). Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *Acacia auriculiformis*. Biol. Fert. Soils, 38: 170–175.

Giovannetti M, Mosse B (1980). An evaluation of techniques to measure vesicular-arbuscular infection in roots. New Phytol., 84: 489-500.

González-Núñez LM, Tóth T, García D (2004). Integrated management for the sustainable use of salt-affected soils in Cuba. Integrated management for sustainable use of salt-affected soils in Cuba. Sci. Univ., 20(40): 85-102.

Hartmond U, Schaesberg NV, Graham JH, Syverten JP (1987). Salinity and flooding stress effects on mycorrhizal and non mycorrhizal citrus rootstock seedlings. Plant Soil, 104: 37–43.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000). Plant cellular and molecular responses to high salinity. Annual Review of Plant Physiology. Plant Mol. Biol., 51: 463–499.

Heenan DP, Lewin LG, McCaffery DW (1988). Salinity tolerance in rice varieties at different growth stages. Aust. J. Exp. Agric., 28: 343–349.

Herrera-Peraza RA, Furrazola E, Ferrer RL, Fernández R, Torres Y (2004). Functional strategies of root hairs and arbuscular mycorrhiza in an evergreen tropical forest, Sierra del Rosario, Cuba. Revista CNIC. Biol. Sci., 2: 113-123.

Hildebrandt U, Janetta K, Ouziad F, Renne B, Nawrath K, Bothe H (2001). Arbuscular mycorrhizal colonization of halophytes in Central European salt marshes. Mycorrhiza, 10: 175–183.

Hirrel MC (1981). The effect of sodium and chloride salts on the germination of *Gigaspora margarita*. Mycol., 43: 610–617.

Ipsilantis I, Sylvia DM (2006). Abundance of fungi and bacteria in a nutrient-impacted Florida wetland. Appl. Soil Ecol., 35: 272–280.

Ipsilantis I, Sylvia DM (2007). Interactions of assemblages of mycorrhizal fungi with two Florida wetland plants. Appl. Soil Ecol., 35: 261–271.

Jeon WT, Park CY, Cho YS, Park KD, Yun ES, Kang U G, Park ST, Choe ZR (2004). Root growth characteristics of rice grown under long-term fertilization of chemical fertilizer and compost in paddy. Korea J. Crop Sci., 48: 484-489.

Juniper S, Abbott L (1993). Vesicular–Arbuscular mycorrhizas and soil salinity. Mycorrhiza, 4: 45–57.

Khan AG (1993). Occurrence and importance of mycorrhizae in aquatic trees of New South Wales, Australia. Mycorrhiza, 3: 31–38.

Khan AG, Belik M (1995). Occurrence and ecological significance of mycorrhizal symbiosis in aquatic plants. In: Varma A and Hock B (Eds.), Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology. Springer-Verlag, Berlin, pp. 627–666.

Keren R (2000). Salinity. In: Sumner, M.E. (Ed.), Handbook of Soil Science. CRC Press, Boca Raton, pp. G3–G25.

Khatun S, Rizzo CA, Flowers TJ (1995). Genotypic variation in the effect of salinity on fertility in rice. Plant Soil, 173: 239–250.

Kozlowski TT, Pallardy SG (1984). Effect of flooding on water, carbohydrate and mineral relations. In: Kozlowski TT (Ed) Flooding and plant growth. Academic Press, London, pp: 165–193.

Ladha JK, Bruijin FJ, Malik KA (1997). Introduction: Assessing opportunities for nitrogen fixation in rice a frontier project. Plant Soil, 194: 1–10.

Landwehr M, Hildebrandt U, Wilde P, Nawrath K, Toth T, Biro B, Bothe H (2002). The arbuscular mycorrhizal fungus *Glomus geosporum* in European saline, sodic and gypsum soils. Mycorrhiza. 12: 199–211.

Liang Y, Nikolic M, Peng Y, Chen W, Jiang Y (2005). Organic manure stimulates biological activity and barley growth in soil subject to secondary salinization. Soil Biol. Biochem., 37: 1185–1195.

Llonin D, Medina N (2002). Mineral nutrition with N, P and K in the symbiotic mycorrhizal fungi, tomato (*Lycopersicon esculentum* Mill) in Ferralsol. Trop. Crops, 23: 83-88.

Maggio A, De Pascale S, Angelino G, Ruggiero C, Barbieri G (2004). Physiological response of tomato to saline irrigation in long-term salinized soils. Eur. J. Agron., 21: 149–159.

Mc Millen BG, Juniper S, Abbott LK (1998). Inhibition of hyphal growth of a vesicular–arbuscular mycorrhizal fungus in soil containing sodium chloride limits the spread of infection from spores. Soil Biol. Biochem., 30: 1639–1646.

Miller SP, Bever JD (1999). Distribution of arbuscular mycorrhizal fungi in stands of the wetland grass *Panicum hemitomon* along a wide hydrologic gradient. Oecologia, 119: 586–592.

Miller S (2000). Arbuscular mycorrhizal colonization of semi-aquatic grasses along a wide hydrologic gradient. New. Physiol., 145: 145-155.

Minagri (1999). Rice Research Institute. Technical Instructions Rice. Havana, Cuba, p. 119.

Munns R, Schachtman DP, Condon AG (1995). The significance of a two-phase growth response to salinity in wheat and barley. Aust. J. Plant Physiol., 22: 561–569.

Nourbakhsh F, Sheikh-Hosseini AR (2006). A kinetic approach to evaluate salinity effects on carbon mineralization in a plant residue-amended soil. J. Zhejiang Univ. Sci. B., 7(10): 788-793.

Pfeiffer CM, Bloss HE (1988). Growth and nutition of guayule (*Parthenium argentatum*) in a saline soil as influenced by vesicular–arbuscular mycorrhiza and phosphorus fertilization. New Phytol., 108: 315–321.

Phillips DM, Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit.. Mycol. Soc., 55: 158-161.

Pérez-Ortega EJ (2010). Arbuscular mycorrhizal fungi (AMF) for the biosecurity of pathogensin tomato crop (*Solanum lycopersicum* L.). PhD. Thesis. Havana University, Faculty of Biology, National Institute of Agricultural Sciences. Havana, Cuba, p. 138.

Rao DLN (1998). Biological amelioration of salt-affected soils. In: Microbial Interactions in Agriculture and Forestry. Science Publishers, Enfield, USA, 1: 21–238.

Ragupathy S, Mohankumar V, Mahadevan A (1990). Occurrence of vesicular-arbuscular mycorrhizae in tropical hydrophytes. Aquat. Bot., 36: 287-291.

Rivera RA, Fernández F (2005). Inoculation and management of mycorrhizal fungi within tropical agroecosystems. In: Uphof N (Ed), Biological approaches to sustainable soil systems. Cornell University, New York, pp. 479-488.

Rosendahl CN, Rosendahl S (1991). Influence of vesicular arbuscular mycorrhizal fungi (*Glomus* spp.) on the response of cucumber (*Cucumis sativis* L.) to salt stress. Environ. Exp. Bot., 31: 313–318.

Ruiz-Lozano JM, Azcón R (2000). Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal *Glomus* sp. from saline soils and *Glomus deserticola* under salinity. Mycorrhiza, 10: 137–143.

Ruiz-Lozano JM, Azcón R, Gómez M (1996). Alleviation of salt stress by arbuscular-mycorrhizal *Glomus* species in *Lactuca sativa* plants. Physiol. Plantarum, 98: 767–772.

Ruiz L (2001). Effectiveness of mycorrhizal associations on plant roots and tubersin brown soils with carbonates and Reds Ferrallitic central Cuba. Thesis in Science Degree option to Doctor in Agricultural Sciences. INCA, p. 117.

Sánchez C (2001). Use and management of mycorrhizal fungi and green manures in the production of coffee seedlings in some soils of Guamuhayasolid."Thesis in Science Degree option to D octor in Agricultural Sciences. INCA, p. 105.

Sánchez-Blanco MJ, Conejero W, Navarro A, Ortuño MF, Alarcón JJ, Torrecillas A, Morte A, García-Mina JM, López LF (2004). Implementation of a new mycorrhizal inoculant fluid through drip irrigation on lettuce. Agric. Murcia, 3: 14-15.

Sannazzaro AI, Ruiz AO, Albertó EO, Menéndez AB (2006). Alleviation of salt stress in *Lotus glaber* by *Glomus intraradices*. Plant Soil, 285: 279–287.

Sass RL, Cicerone RJ (2002). Photosynthate allocations in rice plants: Food production or atmospheric methane? PNAS, 99: 11993–11995.

Secilia J, Bagyaraj DJ (1992). Selection of efficient vesicular-arbuscular mycorrhizal fungi for wetland rice. Biol. Fert. Soils, 13: 108-111.

Shangguan ZP, Shao MA, Ren SJ, Zhang LM, Xue Q (2004). Effect of nitrogen on root and shoot relations and gas exchange in winter wheat. Bot. Bull. Acad. Sin., 45: 49–54.

Sharifi M, Ghorbanli M, Ebrahimzadeh H (2007). Improved growth of salinity-stressed soybean after inoculation with salt pre-treated mycorrhizal fungi. J. Plant Physiol., 164: 1144-1151.

Singh RP, Choudhary A, Gulati A, Dahiya HC, Jaiwal PK, Sengar RS (1997). Response of plants to salinity in interaction with other abiotic and factors. In: Jaiwal PK, Singh RP and Gulati A (Eds.), Strategies for Improving Salt Tolerance in Higher Plants. Science Publishers, Enfield, USA, pp. 25–39.

Smith FA, Smith SE (1997). Structural diversity in (vesicular)–arbuscular mycorrhizal symbioses. New Phytol., 137: 380–388.

Stevens KJ, Peterson RL (1996). The effect of a water gradient on the vesicular-arbuscular mycorrhizal status *of Lythrum salicaria* L. (purple loosestrife). Mycorrhiza, 6: 99–104.

Terry E, Pino MA and Medina N (1998). Agronomic effectiveness of Azofert and Comic in tomato crop (*Lycopersicon esculentum* Mill). Cult. Trop., 19: 33-37.

Tian C, Feng G, Li XL, Zhang FS (2004). Different effects of arbuscular mycorrhizal fungal isolates from, saline or non-saline soil on salinity tolerance of plants. Appl. Soil Ecol., 26: 143–148.

Thormann MN, Currah RS, Bayley SE (1999). The mycorrhizal status of the dominant vegetation along a peatland gradient in southern boreal Alberta, Canada. Wetlands, 19: 438–450.

Trouvelot A, Kough J, Gianinazzi-Pearson V (1986). Measuring the rate of VA mycorrhization of root systems. Research Methods for Estimating havinga functional significance. In: Gianinazzi-Pearson V and Gianinazzi S (Eds), Proceedings of the 1st European Symposium on Mycorrhizae: Physiological and Genetical Aspects of Mycorrhizae, Dijón, INRA, Paris, pp. 217-222.

Turner SD, Amon JP, Schneble RM, Friese CF (2000). Mycorrhizal fungi associated with plants in ground-water fed wetlands. Wetlands, 20: 200–204.

UNCTAD Secretariat from the Food and Agriculture Organization of the United Nations (FAO, 2008).

Yano-Melo AM, Saggin OJ, Costa MJr. L (2003). Tolerance of mycorrhized banana (*Musa sp. cv. Pacovan*) plantlets to saline stress. Agric. Ecosyst. Environ., 95: 343–348.

Yuang B, Li Z, Liu H, Gao M, Zhang Y (2007). Microbial biomass and activity in salt affected soils under arid conditions. Appl. Soil Ecol., 35: 319–328.

Zeng L, Shannon MC (2000). Salinity Effects on Seedling Growth and Yield Components of Rice. Crop Sci., 40: 996–1003.

Zhang DY (1995). Analysis of growth redundancy of crop root system in semi-arid area. Act Bot, Boreali-Occidentalia Sin., 15: 110–114.

Zhang Q, Wang G (2005). Studies on nutrient uptake of rice and characteristics of soil microorganisms in a long-term fertilization experiments for irrigated rice. J. Zhejiang Univ. Sci., 68: 147-154.

Zhu JK (2002). Salt and drought stress signal transduction in plants. Ann. Rev. Plant Biol., 53: 247–273.